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Changes in oil, sugars and nitrogenous components during germination of sunflower seeds, *Helianthus annuus*

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Abstract. Since germination has been found to improve the nutritional quality of grains, sunflower seeds *Helianthus annuus* were germinated for up to five days, and the cotyledons were analyzed for oil, sugar, free amino acid, non-protein nitrogen, lysine, tryptophan and methionine contents. Protein was fractionated. Soaking and germination increased the non-protein nitrogen, total free amino acid, lysine and tryptophan contents. Protein content and dry weight decreased. The oil content decreased significantly after 72 hours of germination. The reducing sugars increased gradually until day 'five' of germination.The saline soluble albumin and globulin fractions decreased while the glutelin content increased during germination. No changes were noticed in the prolamin and methionine contents.

Key words: Aminoacids, Germination, *Helianthus annuus*, Oil, Protein fractions, Sugars

Introduction

Sunflower is one of the major oil seed crops and ranks second as a source of vegetable oil in the world. It is also a good source of proteins. Sunflower proteins comprise about 15–20% of the whole seed and 40% of the dehulled oil meal [1, 2]. Though sunflower meal is mainly marketed in animal feeds, it is suitable for use in human food products also because sunflower proteins have good nutritional and functional properties [3–5]. However, the lysine content of sunflower protein is very low and insufficient [2]. Thus, an increase in lysine content would greatly improve its quality and utility. Germination is one of the means of improving the quality. Hence, the objective of this study was to evaluate the effect of germination on the lysine and tryptophan contents and on the protein profile of sunflower seeds.

Table 1. Changes in the dry matter, oil, sugar and nitrogenous components of the cotyledon of sunflower seeds, *Helianthus annuus* Var. Co 1

Days of germination	Cotyledon dry matter[1] (%)	Oil[2] (%)	Total protein[3] (%)	NPN (%)	Total free amino acids[3] (%)	Total sugars[3] (%)	Reducing sugars[3] (%)
0	$67.4^{a} \pm 0.30$	$50.04^{a} \pm 0.48$	$49.75^{a} \pm 0.41$	$0.63^{a} \pm 0.01$	$0.43^{a} \pm 0.01$	$7.08^{a} \pm 0.11$	$0.54^{a} \pm 0.08$
1	$62.4^{b} \pm 0.14$	$49.00^{a} \pm 0.68$	$48.13^{b} \pm 0.42$	$1.26^{b} \pm 0.01$	$0.87^{b} \pm 0.01$	$5.13^{b} \pm 0.04$	$0.90^{b} \pm 0.06$
2	$58.2^{c} \pm 0.07$	$48.40^{a} \pm 0.16$	$40.94^{c} \pm 0.30$	$2.10^{c} \pm 0.08$	$1.42^{c} \pm 0.09$	$3.21^{c} \pm 0.06$	$1.16^{c} \pm 0.06$
3	$56.8^{d} \pm 0.07$	$47.90^{a} \pm 0.38$	$39.31^{d} \pm 0.77$	$2.20^{c} \pm 0.01$	$1.73^{d} \pm 0.03$	$4.81^{d} \pm 0.07$	$1.20^{c} \pm 0.06$
4	$50.9^{e} \pm 0.03$	$41.00^{b} \pm 0.77$	$38.63^{d} \pm 0.29$	$2.20^{c} \pm 0.07$	$2.15^{e} \pm 0.05$	$6.97^{e} \pm 0.10$	$2.58^{d} \pm 0.11$
5	$49.1^{f} \pm 0.07$	$33.30^{c} \pm 0.42$	$34.81^{e} \pm 0.74$	$2.33^{e} \pm 0.03$	$5.51^{f} \pm 0.14$	$7.32^{f} \pm 0.03$	$4.40^{e} \pm 0.15$
Critical difference ($p = 0.05$)	0.45	3.58	0.96	0.36	0.20	0.21	0.26

[1] Percent of non-decorticated seed.
[2] Percent of decorticated seed.
[3] Percent of dehulled and defatted seed.
Each value represents the mean of quadruplicate analyses ± standard deviation. In the same column, values followed by the same superscripts are not significantly different at 5% level.

Table 2. Changes in the total nitrogen, lysine and tryptophan contents during germination of sunflower seeds, *Helianthus annuus* Var. Co 1

Days of germination	Total N content (%)	Lysine (g/16 g N)	Tryptophan (g/16 g N)
0	$8.59^{a} \pm 0.07$	$2.68^{a} \pm 0.06$	$0.65^{a} \pm 0.01$
1	$8.96^{a} \pm 0.15$	$3.27^{a} \pm 0.19$	$1.62^{b} \pm 0.01$
2	$8.65^{a} \pm 0.18$	$3.98^{b} \pm 0.01$	$2.66^{c} \pm 0.04$
3	$8.49^{a} \pm 0.18$	$6.31^{c} \pm 0.13$	$2.81^{d} \pm 0.09$
4	$8.38^{ab} \pm 0.33$	$8.15^{d} \pm 0.48$	$4.61^{e} \pm 0.06$
5	$7.90^{b} \pm 0.34$	$11.90^{e} \pm 0.02$	$7.77^{f} \pm 0.02$
Critical difference ($p = 0.05$)	0.49	0.68	0.051

Each value represent the mean of quadruplicate analyses ± standard deviation. In the same column, values followed by the same superscripts are not significantly different at 5% level.

Materials and methods

The sunflower *Helianthus annuus*, variety Co 1, developed at the Tamil Nadu Agricultural University was selected for the study. The seeds were obtained from the Oil Seeds Breeding section, Millet breeding station, Tamil Nadu Agricultural University. The seeds were surface sterilized with 1% sodium hypochlorite solution followed by soaking in 70% ethanol for about 3 min. The seeds were then rinsed thoroughly with distilled water and soaked for about 3 hours in distilled water. The imbibed seeds were spread evenly on layers of wet filter paper kept in large petri dishes (four replications per sample) and allowed to germinate at room temperature for 1 to 5 days in the dark. The filter papers were moistened at regular intervals. Samples were collected at 24-hour intervals from each of the replica plates and used for further analyses. After removing the hulls, the cotyledons were dried and a portion was then used for oil estimation [6]. The remaining cotyledons were ground and defatted in hexane as described by Raymond et al. [7]. The defatted meal was further ground in a mortar and pestle and passed through an 80 mesh sieve. The powder was used for the remaining analyses.

The saline soluble proteins were extracted from 500 mg of defatted meal using 0.02 M Tris-borate buffer (pH 8.6) containing 10% (w/v) sodium chloride [8]. Supernatants from three successive extractions were collected and made up to 50 ml. The alcohol soluble proteins were extracted from the residue after the above extraction, and the alkali soluble proteins were extracted from the residue after alcohol extraction following the method of Køie and Nielsen [9].

Table 3. Changes in the protein profile during germination of sunflower seeds *Helianthus annuus*, Var. Co 1

Days of germination	Total extractable protein nitrogen (%)	Salt soluble protein nitrogen (%)	Alcohol soluble protein nitrogen (%)	Alkali soluble protein nitrogen (%)
0	$6.99^{a} \pm 0.13$	$5.05^{a} \pm 0.13$	$0.833^{a} \pm 0.02$	$1.11^{a} \pm 0.04$
1	illegible	illegible	illegible	illegible
2	$6.08^{b} \pm 0.06$	$4.03^{b} \pm 0.14$	$0.834^{a} \pm 0.03$	$1.22^{ab} \pm 0.13$
3	$5.87^{b} \pm 0.01$	$3.71^{bc} \pm 0.01$	$0.833^{a} \pm 0.08$	$1.33^{b} \pm 0.08$
4	illegible	illegible	illegible	illegible
5	illegible	illegible	$0.626^{b} \pm 0.06$	$1.33^{b} \pm 0.10$
Critical difference ($p = 0.05$)	0.79	0.38	0.012	0.211

Each value represent the mean of quadruplicate analyses ± standard deviation. In the same column, values followed by the same superscripts are not significantly different at 5% level.

The total protein and the protein content in the different protein fractions were obtained by the microKjeldahl procedure [10] following the recommendations given by the Swedish Seed Association (1976). The total free amino acid content was determined by the method of Misra et al. [11]. Lysine and tryptophan contents were estimated by the methods of Vogel and Shimura [12] and Friedman and Finely [13], respectively. Non-protein nitrogen was estimated by the microKjedahl method in the supernatant of TCA extracts of the defatted meal following the method of Singh et al. [14]. Methionine content was determined by the method of Horn et al. [15].

Total sugars and reducing sugars were estimated in an 80% ethanol extract of the dehulled, defatted meal. Phenol-sulphuric acid [16] and Nelson-Somogyi reagent [17], respectively were used.

Data were analyzed using analysis of variance. Significance was accepted at $p \leq 0.05$ level.

Results and discussion

The changes in dry matter, oil and protein as percent (w/w) of the cotyledon of sunflower seed during germination and early growth are presented in Table 1. The cotyledon content decreased from 67.4% on day zero to 49.1% on day five indicating mobilization of reserves to the growing axis.

The decorticated seeds had an initial oil content of 50.4% on day zero which was depleted to 33.3% by day five. However, the oil depletion rate was not significant until 72 hours after imbibition. Similar reports indicate that

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the storage triglycerides are not utilized over the first 2 days after imbibition in castor beans [18].

The initial extractable protein content was 43.7% in the dehulled and defatted meal. The protein content decreased significantly on day one. Simultaneously, the non-protein nitrogen and total free amino acid contents increased (Table 1). Lawrence & Grant [19] have reported an increase in the free amino acid content in the germinating seedlings of peas.

The total sugar content decreased until day two after which there was an increase until day five (Table 1). The free fatty acids produced during lipolysis in oil seeds do not accumulate but are rapidly degraded and converted to carbohydrates and transported to the growing regions of the seedlings [18]. The decrease in the content of total sugars until day two may be due to transport to the growing axis coupled with the non degradation of triglycerides until day two.

The content of reducing sugars increased gradually from day zero to day five. The main product of metabolism during germination in sunflower seeds is glucose [20], not sucrose. This is in contrast to other oil seeds like peanuts and castor beans where the main product of metabolism during germination is sucrose.

Sunflower protein is well balanced in all essential amino acids except lysine [21]. In the germinated samples of sunflower, large increases in lysine (2.7 to 11.9 g/16 g N) and tryptophan (0.7 to 7.8 g/16 g N) contents were found (Table 2). Wu & Wall [22], Balasubramanian & Sadasivam [23] and Parameswaran & Sadasivam [24] have reported increases in lysine and tryptophan contents proteins during germination of sorghum, grain amaranth and prosomillet, respectively. Increases in lysine and tryptophan contents during germination of maize seeds have been reported by Tsai et al. [25] and Chibber et al. [26]. There was no significant change in methionine content of the cotyledons during germination (data not shown).

The contents of the different protein fractions of the cotyledons at various stages of germination are summarized in Table 3. The albumin and globulin (saline soluble) fractions continued to decrease while the glutelins(alkali soluble) increased. There was no significant change in the contents of prolamins(alcohol soluble) until day four. Although a decrease in the total contents of albumins and globulins was noticed, *de novo* synthesis of enzyme proteins (albumins) and increased content of high lysine glutelins may contribute to the increased lysine content.

Sunflower kernels and their defatted meal have several advantages over other oil seed meals as a human protein food. These include flavor, the absence of antinutritional or toxic factors, and high digestibility and biological value [27]. In general, sprouted seeds are reported to have a higher nutritional

quality than raw seeds [28]. In the present study, sprouted sunflower seeds (24 hours after imbibition) had a 20% increase in lysine content and a significant ($p = 0.05$) increase in tryptophan content with no significant decrease in the oil and protein contents. Thus, the sprouted sunflower seeds had higher nutritional quality than the raw seeds.

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